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ORIGINAL ARTICLE

Major Factors Affecting the Distribution of Anuran Communities in the Urban, Suburban and Rural Areas of Shanghai, China

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Abstract There is a dearth of information on the effects of landscape and microhabitat variables on the distribution of anurans in areas of rapid urban development, in both tropical and subtropical regions. Therefore, we studied 24 wetlands sites from the center of Shanghai city, China extending outward to rural areas. Sampling was performed from May through July 2014. Urbanization was categorized by the proportion of hard ground cover. Transect sampling and 'calling' surveys were used to investigated the richness and density of anurans; microhabitat factors were recorded simultaneously. One-way analysis of variance and Kruskal–Wallis tests were conducted to analyze differences of total density, species richness and density of individual anuran species in the three urbanization levels; redundancy analysis was carried out on the relationship between anuran density and environmental variables. Species richness was lowest in the areas where the proportion of hard ground cover was > 80%, and the total density of anurans was highest in the areas where coverage of the hard ground cover was < 30%. We recorded five species belonging to four genera and four families and an individual anuran species that had varied representations in urban environments. Beijing gold-striped pond frogs (*Pelophylax plancyi*) and Zhoushan toads (*Bufo gargarizans*) appeared to be well adapted to the Shanghai metropolis. Large water environments and aquatic vegetation (floating-leaves and emergent vegetation) were indicators of the presence of Beijing gold-striped pond frogs. The density of black-spotted pond frog (*Pelophylax nigromaculatus*) was at the lowest density in the areas where hard ground coverage was > 80%, and tended to prefer larger bodies of water. Hong Kong rice-paddy frogs (*Fejervarya multistriata*) and ornamented pygmy frogs (*Microhyla achatina*) both suffered severely from cropland loss due to urban development. Bare land around breeding grounds was important for Hong Kong rice-paddy frogs, since it usually chooses mud coast caves for hibernation.

Keywords subtropical regions, rapid urbanization, amphibian, habitat variable, breeding seasons

1. Introduction

Anurans are indicator species of the health of wetland ecosystems (Guzy *et al.*, 2012), and are facing worldwide decline. The speed at which this decline in species richness and abundance is occurring is faster than that of birds and mammals (Stuart *et al.*, 2004). This downtrend has happened for many reasons, with urban development being recognized as one of the main causes.

Urban development changes land-use patterns, increasing impervious surface cover with the widespread development of buildings and extensive complex road networks (McDonnell *et al.*, 1997; Collins *et al*. 2000; McKinney, 2002), resulting in the fragmentation and loss of vital habitats and habitat corridors (Czech *et al*., 2000; McKinney, 2002; Miller and Hobbs, 2002). Complex road systems also cause a high mortality of anurans (Hels and Buchwald, 2001; Mazerolle, 2004). Anurans usually form metapopulations in urban environments, therefore, habitat connectivity is vital to their survival (Brown and Kodric-

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Brown, 1977), as their ability to migrate is relatively poor. All the above urbanization factors have a negative influence on amphibian species richness and anuran migration (Vos and Chardon, 1998; Rubbo and Kiesecker, 2005; Pautasso, 2007; Pillsbury and Miller, 2008; Vignoli *et al.*, 2009).

On a microhabitat scale, aquatic and terrestrial habitats are both necessary for anurans to complete their life cycle. Urbanization, however, has led to the degradation of a diverse range of suitable habitats (Dodd and Smith, 2003; Stevens and Paszkowski, 2004). The urban heat island effect which occurs in urban areas has caused an increase not only in atmospheric temperatures but also local water temperatures, resulting in increased mortality rates of amphibian eggs (Bornstein, 1968; McDonnell *et al.*, 1993; Corn and Muths, 2002; Grimm *et al.*, 2008). Urban water environments are prone to heavy metal pollution and natural water are at risk of contamination due to run-off from the wide application of chemical fertilizers and pesticides, threatening larval survival (Semlitsch, 2000; Paul and Meyer, 2001; Boone and Bridges, 2003; Rubbo and Kiesecker, 2005). The habitat characteristics of artificial wetlands, such as the presence of predator fish also negatively affect the survival of breeding amphibians (Orizaola and Braña, 2006).

Only 1% of the research related to organismal distribution across urbanization gradients relates to amphibians and reptiles (McDonnell and Hahs, 2008). Studies on the effect of rapid urbanization on anurans in tropical and subtropical regions are rare (Hamer and McDonnell, 2008). Nearly 40% of China's wetlands are under moderate and serious threat (An *et al.*, 2008). Forest and agricultural areas around cities are being quickly replaced by residential buildings, roads and other constraining features (Fazal, 2000; Liu *et al.*, 2003; Yoon, 2009). Urbanization has developed rapidly in the past few decades in China (Liu *et al.*,2003), especially in Shanghai, which is located along the Yangtze River estuary and is a commercial and financial center of mainland China. The Shanghai region experienced rapid economic and population growth, from 1982 to 1990; the Shanghai downtown area increased from 149.85 km^2 to 279.78 km^2 and continued to expand to 377.56 km^2 after 2000 (Song, 2003). From 1989 to 2013, the population of Shanghai had an average annual growth rate of 0.89%. The population density has grown from 2013 persons/km^2 to 3809 persons/km^2 (Shanghai statistical yearbook, 1989–2013), making it the city with the highest population density and the fastest growing urban development in China (Wang *et al.*, 2014). According to terrestrial wildlife resource surveys in Shanghai, three anuran species disappeared between 2000 and 2013. Dramatic urban development may be responsible for the decline and disappearance of these anuran species (Xie *et al.*, 2002).

Our research selected Shanghai, the largest city with the fastest urbanization in China as the study area, and we investigated the distribution patterns and major influential factors on the anuran community within each urbanization level (urban, suburban and rural) (Matson, 1990; McDonnell and Pickett, 1990), which has been commonly applied to studies of urbanization impacts on avian populations (Clergeau *et al.*, 1998, 2006; Melles *et al.*, 2003; Crooks *et al.*, 2004; Vignoli *et al.*, 2013). This awareness should help implement and support effective protection strategies for amphibians.

2. Materials and Methods

2.1 Study Area The research study area was located in the city of Shanghai, China, a recognized subtropical region, and an area undergoing accelerated urbanization. Urban expansion infrastructure needs to meet a myriad of developmental demands, including new housing, commercial buildings, transport infrastructure, etc. Most researchers use 'land-use' or 'land-cover' as criteria for determining urbanization gradients (McDonnell and Hahs, 2008). This research used the proportion of hard ground cover within 2 km of the sample plot to define urbanization levels (Marzluff *et al.*, 2001). "Hard ground cover" included residential or commercial buildings, roads networks and other impermeable strata, and sites with values > 80% were defined as urban areas, between 30%–80% as suburban areas and < 30% as rural areas. In this study, 24 sites were chosen from the city center to the outer rural perimeter (Figure 1). Eight study sites were located within each urbanization level (urban, suburban and rural). Site selection was based on two principles; first, sites must be at least 1 km apart to control data independence and second, sites had to be classified as a permanent or semi-permanent pond to qualify for investigation and ensure reliable data collection.

2.2 Anuran Sampling During the breeding seasons, May through July 2014, transect sampling (Harris and Burnham, 2002), and calling surveys (Weir *et al.*, 2009) were conducted to evaluate the population size. Due to variation in sample plot accessibility, sample transect lengths were not always consistent. The mean sample transect length was 1113 m (standard error SE: 126 m), with a transect width of 5 m. Surveys were conducted

at least 0.5 h after sunset and completed by 00:00. Air temperatures ranged from 18.7 ºC–26.5 ºC and water temperatures between 22.1 ºC–26.7 ºC, with wind speeds below 5.8 m/s.

2.3 Landscape Characteristics In general, anurans tend to have poor dispersal ability (Semlitsch, 2000; Smith and Green, 2005), based on their limited distribution potential. Therefore, landscape characteristics were quantified within 1 km radius of each sample plot using Google Earth Pro (version 6.2.2.6613), which included water and cropland coverage.

2.4 Breeding Habitat Characteristics As microhabitat variables, we selected water depth, pH and water salinity, aquatic vegetation coverage (floating-leaves vegetaion, emergent vegetation and submerged vegetation), argillaceous bank coverage, slopes and bare land coverage within 2 m of the sample plot. Water depth was measured using a 1.5 m tape, and five water depths were taken within 1 m of the water's edge (Hazell *et al.*, 2004), to obtain an averaged value. The AZ8685 Pen Type pH meter and the AZ8371 handheld salinometer (Frank Electronics Co., Ltd., Shenzhen, China) were used to measure pH and water salinity at the water surface, approximately 30 cm from the water's edge (Hazell *et al.*, 2004; Rubbo and Kiesecker, 2005). Visual estimation was used to determine aquatic vegetation coverage (Price *et al.*, 2005; Clark *et al.*, 2007), argillaceous bank coverage and bare land coverage. The pond slope was measured using a geological compass. Data of breeding habitat variables were collected during anuran surveys.

2.5 Statistical Analyses The Kolmogorov–Smirnov test was used to determine whether total density, species richness and density of targeted anuran species were normally distributed. Data were converted by arcsine transformation in order to conform to a normal distribution with homogeneity of variance.

One-way analysis of variance (ANOVA) followed by a post-hoc Tukey's multiple comparison test was used to deduce differences of the density of Beijing gold-striped pond frogs and Zhoushan toads along the urbanization gradient, because they conformed to the assumed parameters. However, Kruskal–Wallis test, followed by post-hoc pairwise comparison tests were used to analyze density differences of Black-spotted pond frogs, Hong Kong rice-paddy frogs and ornamented pygmy frogs in each urbanization level, because they did not conform the assumed parameters. We conducted this statistical analyses using SPSS version 20.0.

Detrended correspondence analysis (DCA) was used to analyze the data of species-sample,and the gradient lengths of axis 1 were 2.641<3; therefore, we conducted a redundancy analysis (RDA) involving five species and environmental variables to determine which environmental variables were the major influencing factors in the distribution of individual anuran species at breeding sites. RDA is an extension of multiple linear regression (Dodkins *et al.*, 2005), which is a simple method to elucidate the relationship between species and environmental variables. The statistical significance of the RDA was evaluated by Monte Carlo permutation tests. We conducted this statistical analyses in Canoco 4.5.

3. Results

3.1 Distribution of the Anuran Community in the Urban-Suburban-Rural Areas Anuran species richness was significantly lowest in the areas where hard ground coverage was > 80% ($F_{2,21}$=8.749, P=0.002), but there was no significant difference between rural and suburban areas (Table 1). The anuran total density was significantly increased with decreasing urbanization degree ($F_{2,21}$=7.429, P=0.004), and reached its peak where hard ground coverage was < 30% (rural areas), there was no significant difference between urban and suburban areas (Table 1).

Each anuran species was found to have its own individual responses to urbanization. The density of the Hong Kong rice-paddy frogs (Kruskal–Wallis test: χ^2=15.398, df=2, P<0.001) and ornamented pygmy frogs (Kruskal-Wallis test: χ^2=6.530, df=2, P=0.038) was significantly different between the three urbanization levels (Table 1); the density of Hong Kong rice-paddy frogs increased with decreasing urbanization degree and ornamented pygmy frogs were not present in the urban areas. The density of Black-spotted pond frogs were significantly the lowest in the areas where coverage of the hard ground cover was > 80% (urban areas) (Kruskal–Wallis test: χ^2=6.935, df=2, P=0.031), but no significant difference was noted between the suburban and rural areas (Table 1).

The density of the Beijing gold-striped pond frogs ($F_{2,21}$=3.419, P=0.052) and Zhoushan toads ($F_{2,21}$=0.340, P=0.716) did not reveal a significant difference between three urbanization levels (Table 1).

3.2 Factors Underlying Anuran Community Distribution In the RDA analyses, the four axis explained 60.7% of the species variation. The percent of explained variation for the first and second axis of RDA was 35.7% and 15.0%, respectively. Correlation between

environmental variables, ordering axis and species variable ordering axis was 0.922 and 0.894, respectively (Table 2). According to Monte Carlo permutation tests of the RDA, explained variation of the first canonical axis had a significant effect on species (F=6.657, P=0.018), and all canonical axis was also have significant (F=1.773, P=0.039).

Water coverage, cropland coverage, emerged vegetation coverage and bare land coverage were significantly correlated to the first axis (Table 3, Figure 2), these environmental variables increased along the axis one from left to right; water coverage and bare land coverage were significantly correlated to the second axis (Table 3, Figure 2), and water coverage was increased along axis two from the bottom up; however, bare land was decreased along axis two from the bottom up.

Our results indicated that anurans had species-specific requirements for habitat conditions and resources. The result of RDA shows that Black-spotted pond frogs were significantly positively associated with water coverage (Table 4, Figure 2), Beijing gold-striped pond frogs were positively associated with water coverage and aquatic vegetation coverage including floating-leaves and emerged vegetation (Table 4, Figure 2), cropland coverage and bare land coverage had significant influence on the density of Hong Kong rice-paddy frogs (Table 4, Figure 2), and only cropland coverage had a significantly relationship with Ornamented pygmy frogs (Table 4, Figure 2). RDA analysis did not show any variable that significantly influenced the distribution of Zhoushan toads.

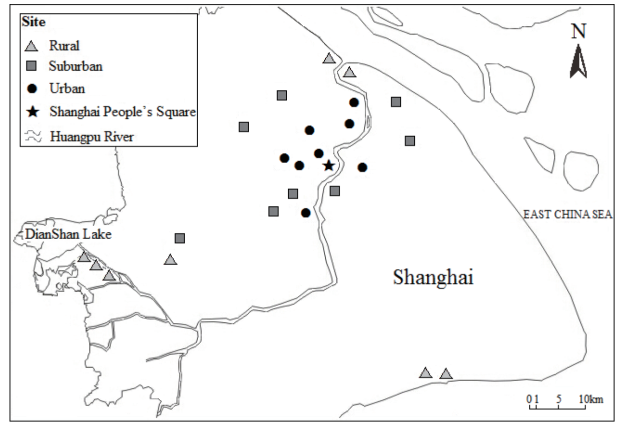

Figure 1 Location of study sites in Shanghai. The People's square in Shanghai was served as the urban center in this study.

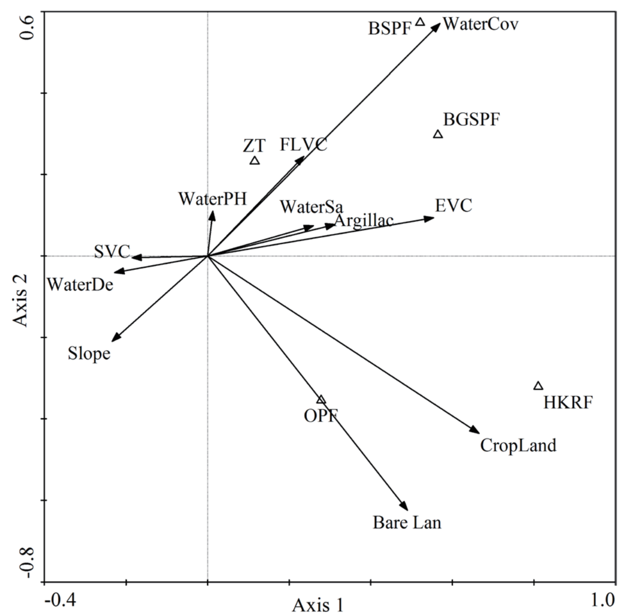

Figure 2 First and second axis of the RDA involving anuran species and environmental variables in Shanghai, Environmental variables (arrows) are: Water Coverage (WaterCov); Cropland Coverage (Cropland); Water depth (WaterDe); Slope; Water PH; Water salinity (WaterSa); Floating-leaves vegetation Coverage (FLVC); Emergent vegetation Coverage (EVC); Submerged vegetation Coverage (SVC); Argillaceous bank Coverage (Argillac); Bare land (Bare Lan). Species (acronyms) are: Black-spotted pond frog (BSPF); Beijing gold-strip pond frog (BGSPF); Hong Kong rice-paddy frog (HKRF); Ornamented pygmy frog (OPF); Zhoushan toad (ZT).

4. Discussion

This study found that a high level of urbanization has a negative effect on density and distribution of local anurans (Table 1); similar findings have been reported in other localities (Shirose *et al.* 2000; MacGregor-Fors *et al.*, 2013). As urbanization levels increased, species richness decreased, and total species density reached a minimum where hard ground cover was >80%. Urbanization reduces habitat availability for anurans, and the decreased species richness in urban wetlands was attributable to the rarity of Hong Kong rice-paddy frogs and Ornamented pygmy frogs, which prefer cropland habitat (Table 4), we hypothesize that the disappearance of cropland habitat is the main reason for decline in species richness urban areas.

Individual anuran species have different responses to urbanization, and the influence of urbanization on anurans is highly associated with the life history of the species and their sensitivity to environmental changes (Garden *et al.*, 2006). Decreasing cropland coverage is the main limiting factor affecting the distribution of Hong Kong rice-paddy frogs and Ornamented pygmy frogs in the three urbanization levels (Table 4). This is consistent with the dispersal-dependent-decline hypothesis that

Table 1 Species richness and anuran density in the three urbanization levels.

Species/Parameters	Urbanization levels		
	Urban-Suburban (*P*)	Suburban-Rural (*P*)	Rural-Urban(*P*)
Black-spotted pond frog (*P. nigromaculatus*)[a]	0.036	0.462	0.015
Hong Kong rice-paddy frog (*F. multistriata*)[a]	0.008	0.027	<0.001
Ornamented pygmy frog (*M. ornata*)[a]	0.02	0.826	0.011
Beijing gold-strip pond frog (*P. plancyi*)[b]	0.1	0.982	0.071
Zhoushan toad (*B. gargarizans*)[b]	0.801	0.99	0.721
Total density[b]	0.053	0.41	0.003
Anurans species richness[b]	0.019	0.553	0.002

[a] Values are based on post-hoc pairwise comparison test.
[b] Values are based on post-hoc Tukey's multiple comparison test.

Table 2 Statistical characteristics of four ordination axis of the RDA.

	axis 1	axis 2	axis 3	axis 4
Eigenvalues	0.357	0.15	0.052	0.048
Species-environment correlations	0.922	0.894	0.623	0.494
Cumulative percentage variance of species data	35.7	50.7	55.9	60.7
Cumulative percentage variance of species-environment relation	57.6	81.9	90.3	98.1

Table 3 Correlation coefficients between environmental variation and RDA ordination axis.

	Axis 1	Axis 2	Axis 3	Axis 4
Water Coverage (%)	**0.5253**[*]	**0.5109**[*]	0.0904	–0.043
Cropland Coverage (%)	**0.6137**[**]	–0.3891	0.2043	0.1173
Water depth	–0.2099	–0.0362	–0.225	–0.1592
Slopes	–0.216	–0.1871	–0.123	–0.2441
Water PH	0.0122	0.0989	–0.2269	–0.0441
Water salinity	0.2394	0.0666	0.0349	–0.1
Floating–leaves vegetation (%)	0.2175	0.2191	–0.3404	0.2567
Emergent vegetation (%)	**0.5112**[*]	0.0843	0.1375	0.2275
Submerged vegetation (%)	–0.1706	–0.0034	–0.1627	0.0456
Argillaceous bank Coverage (%)	0.288	0.0694	0.1564	0.2785
Bare land Coverage (%)	**0.4522**[*]	**–0.5585**[**]	0.0505	–0.0365

[*] $P<0.05$; [**] $P<0.01$

Table 4 Correlation coefficients between environmental variables and five anurans species.

	BSPF	BGSPF	HKRF	OPF	ZT
Water Coverage (%)	**0.6695**[***]	**0.4314**[*]	0.2751	0.0076	0.181
Cropland Coverage (%)	0.1213	0.2559	**0.6296**[**]	**0.5583**[**]	0.0618
Water depth	–0.1475	–0.1514	–0.124	–0.2722	–0.2355
Slopes	–0.2081	–0.3481	–0.0398	–0.2568	–0.0951
Water PH	0.0145	0.0925	0.0108	–0.2301	–0.0364
Water salinity	0.2426	0.1234	0.1795	0.0816	–0.1593
Floating–leaves vegetation (%)	0.0719	**0.5232**[*]	0.0902	–0.1236	0.0886
Emergent vegetation (%)	0.295	**0.4278**[*]	0.3699	0.3072	0.3195
Submerged vegetation (%)	–0.1807	–0.0444	–0.1201	–1.99	0.1024
Argillaceous bank Coverage (%)	0.1592	0.3393	0.1506	0.3264	0.209
Bare land Coverage (%)	–0.0763	0.0417	**0.6031**[**]	0.3677	–0.0725

[*] $P<0.05$; [**] $P<0.01$; [***] $P<0.001$

Species (acronyms) are: Black-spotted pond frog (BSPF); Beijing gold-strip pond frog (BGSPF); Hong Kong rice-paddy frog (HKRF); Ornamented pygmy frog (OPF); Zhoushan toad (ZT).

sedentary species can be extremely sensitive to habitat loss and habitat degradation, and paddy-associated frogs are sensitive to the loss of cropland habitats (Tsuji *et al.*, 2011). From our research alone, it has been demonstrated that the rapid development of urban infrastructure and shrinkage of cropland reduce the optimum habitat of the Hong Kong rice-paddy frogs and ornamented pygmy frogs, causing their populations to undergo a dramatic decline. These may eventually become the first two threatened anuran species in the next decade. deMaynadier and Hunter (1999) showed that high-density vegetation growing on the edge of wetlands can provide food, shelter and migration and overwintering sites. However, bare land plays an important role in the survival of the Hong Kong rice-paddy frog (Table 4), since it can provide mud caves for hibernation sites (Wang *et al.*, 2008). In the field, we also observed that frogs hide in the mud caves when threatened.

High levels of urbanization hindered the distribution of the Black-spotted pond frog, and reduce their density. However, different from a similar study in Osaka-kobe (Japan) (Tsuji *et al.*, 2011), our research noted that in Shanghai, the density and distribution of the Black-spotted pond frog was restricted by the size of the water area, which is important for their reproduction and larval development (Vignoli *et al*., 2009). Farmland ecosystems become restrictive habitat factors for the survival of Black-spotted pond frog in Osaka-Kobe (Tsuji *et al.*, 2011). Differences in habitat selection may be associated with the different urban development patterns and local agricultural practices. Osaka-Kobe is a metropolitan area with paddy fields widely distributed in rural and urban zones (Tsuji *et al.*, 2011), but the urbanization development of Shanghai is mainly diffused outward in a circular configuration, with no paddy fields in the interior urban environment. Different urbanization development models may contribute to different urbanization pressures on the same anurans, and Black-spotted pond frogs may change its habitat preferences in order to adapt to these different environmental stresses.

It has been reported that some anurans can adapt to urbanization (Rubbo and Kiesecker, 2005; Tsuji *et al.*, 2011). According to our research, rapid urbanization has had little effect on the density and distribution of the Beijing gold-striped pond frogs and Zhoushan toads (Table 1). The Beijing gold-striped pond frog tended to choose a wetland ecosystem with abundant floating-leaves vegetation, such as water lilies (*Nymphaea odorata*) and duckweed (*Spirodela polyrhiza*). Emergent vegetation, such as the lotus flower (*Nelumbo nucifera*) and bulrush (*Phragmites communis*) were also potential indicators for the presence of the frog (Table 4). Aquatic vegetation is also a good indicator for in predicting anuran occurrence and larvae survival of North American amphibians (Skidds *et al.*, 2007; Purrenhage and Boone, 2009), The density of aquatic vegetation affects all life-cycle stages of Beijing gold-striped pond frogs, providing shelter for larvae, spawning sites for adults and can also be used as a platform for resting and breathing (Egan and Paton, 2004; Skidds *et al.*, 2007; Scheffers and Paszkowski, 2013). In addition, large-area water environmental play an important role in the reproduction and migration of Beijing gold-striped pond frogs.

5. Conservation Implications

Our research noted that highly urbanized areas have significant negative effects on the survival of some anurans, and a reasonable collocation of a variety of habitat characteristics which were beneficial to the frogs complete life history are critical for anurans conservation (Garcia-Gonzalez and Garcia-Vazquez, 2012).

According to our research, the survival of the Hong Kong rice-paddy frogs and Ornamented pygmy frogs was dependent on cropland habitat. Retaining this essential habitat is a critical conservation measures for these two anurans. In addition, terrestrial habitats such as reserves of bare land around artificial wetlands are also important, as they can provide hibernation sites and shelter for Hong Kong rice-paddy frogs.

Large-area wetland ecosystems are more productive for the conservation of the Black-spotted pond frogs and Beijing gold-striped pond frog. The question of whether large ponds should be constructed or multiple small ponds interconnected to form a larger integrated water network is still a topic to be discussed and requires further research.

Ponds in urban areas attract visitors and allow for recreation, but also play an important role in the urban ecosystem and have irreplaceable ecological functions in the conservation of urban anurans (Shirose *et al.*, 2000), providing an alternative permanent habitat for urban adaption. Carrier and Beebee (2003) noted that British urban and suburban areas with many good quality ponds provide excellent conditions for the common frogs (*Rana temporaria*). Maintaining floating-leaves vegetation and emergent vegetation in the urban ponds or establishing aquatic vegetation buffer strips along the shoreline of large lakes would be effective methods for towards the conservation of Beijing gold-striped pond frogs.

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